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Richard E. Ostlund Jr.
Washington University School of Medicine in St. Louis

David Gibson
Washington University School of Medicine in St. Louis

et al

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Featured Article

Technical performance of a novel, fully automated electrochemiluminescence immunoassay for the quantitation of β-amyloid (1–42) in human cerebrospinal fluid

Tobias Bittner[a], Henrik Zetterberg[b,c], Charlotte E. Teunissen[d], Richard E. Ostlund, Jr.,[e], Michael Militello[f], Ulf Andreasson[b], Isabelle Hubeek[d], David Gibson[e], David C. Chu[f], Udo Eichenlaub[a], Peter Heiss[a], Uwe Kobold[a], Andreas Leinenbach[a], Kairat Madin[a], Ekaterina Manuilova[a], Christina Rabe[a], Kaj Blennow[b,*]

[a]*Roche Diagnostics GmbH, Penzberg, Germany*
[b]*Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at University of Gothenburg, Mölndal, Sweden*
[c]*UCL Institute of Neurology, London, UK*
[d]*Clinical Chemistry, VU University Medical Center, Amsterdam, the Netherlands*
[e]*Washington University School of Medicine, St. Louis, MO, USA*
[f]*Covance Central Laboratory Services, Indianapolis, IN, USA*

Abstract

Introduction: Available assays for quantitation of the Alzheimer's disease (AD) biomarker amyloid-beta 1–42 (Aβ [1–42]) in cerebrospinal fluid demonstrate significant variability and lack of standardization to reference measurement procedures (RMPs). We report analytical performance data for the novel Elecsys β-amyloid (1–42) assay (Roche Diagnostics).

Methods: Lot-to-lot comparability was tested using method comparison. Performance parameters were measured according to Clinical & Laboratory Standards Institute (CLSI) guidelines. The assay was standardized to a Joint Committee for Traceability in Laboratory Medicine (JCTLM) approved RMP.

Results: Limit of quantitation was <11.28 pg/mL, and the assay was linear throughout the measuring range (200–1700 pg/mL). Excellent lot-to-lot comparability was observed (correlation coefficients [Pearson's r] >0.995; bias in medical decision area <2%). Repeatability coefficients of variation (CVs) were 1.0%–1.6%, intermediate CVs were 1.9%–4.0%, and intermodule CVs were 1.1%–3.9%. Estimated total reproducibility was 2.0%–5.1%. Correlation with the RMP was good (Pearson's r, 0.93).

Discussion: The Elecsys β-amyloid (1–42) assay has high analytical performance that may improve biomarker-based AD diagnosis.

Keywords: Amyloid; Amyloid-beta 1–42; Alzheimer's disease; Assay; Biomarker; Cerebrospinal fluid; Dementia; Diagnosis; Electrochemiluminescence; Immunoassay; Method comparison; Precision; Repeatability; Reproducibility; Variability

1. Introduction

The incidence of Alzheimer's disease (AD) is expected to triple in both the United States and Europe in the next decades, resulting in a substantially increased health care burden [1,2]. Disease-modifying treatments (DMTs) for AD are in development but none are yet available [1] because of the high attrition rate observed in clinical trials [3]. Importantly, most of the agents in development for AD directly target the amyloid pathology of AD. The development and potential availability of these treatments create the need for reliable diagnostic tests that can accurately identify patients with amyloid pathology, who will likely benefit most from these agents. Availability of a DMT would also

*Corresponding author. Tel.: +46-31-343-1791; Fax: +46-31-343-2426.
E-mail address: Kaj.Blennow@neuro.gu.se

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increase the demand for techniques to improve recognition of AD in clinical practice, particularly in the early stages of the disease (mild cognitive impairment [MCI] due to AD, or prodromal AD). Cognitive symptoms are mild and diffuse, which preclude the possibility of differentiating early, prodromal AD from other causes of MCI.

The most recent diagnostic criteria for AD recommend use of in vivo biomarkers of AD pathology to enhance diagnostic accuracy [4]. Candidate biomarkers include decreased amyloid-beta 1–42 (Aβ [1–42]) in the cerebrospinal fluid (CSF), alone or in conjunction with increased total tau or phosphorylated tau, and increased retention of amyloid-targeting radiotracers detected by positron emission tomography (PET). CSF Aβ (1–42) is altered early in the course of the illness [5,6], and CSF Aβ (1–42) levels correlate well with neuropathologic findings (neuritic plaques) consistent with AD [7,8], as well as with PET-based measurements of amyloid pathology [9–11].

These findings support the use of CSF Aβ (1–42) in research diagnostic criteria. However, issues with currently available CSF Aβ (1–42) assays limit their applicability as diagnostic devices worldwide. These issues include high lot-to-lot variability for enzyme-linked immunosorbent assays (ELISAs) [12], as well as between-laboratory variability associated with differences in laboratory procedures and analytical techniques, as assays are most often run manually [13]. In addition, the historical lack of availability of a reference measurement procedure (RMP) for quantitation of CSF Aβ (1–42) has resulted in difficulties in comparing results across assays [14]. Taken together, these shortcomings prevent the establishment of universal diagnostic cutoffs for CSF biomarkers. Finally, available assays have long incubation times, and as most assays are based on the 96-well plate immunoassay format, for financial reasons, laboratories must await a high enough number of samples to justify analysis. Therefore, turnaround times for test results may be as long as several weeks.

The Elecsys β-amyloid (1–42) assay (Roche Diagnostics) is a novel, fully automated, electrochemiluminescence immunoassay, using the sandwich test principle, for quantitation of Aβ (1–42) in human CSF. In this study, we describe the technical performance of this assay, including its standardization Joint Committee for Traceability in Laboratory Medicine (JCTLM) approved RMP.

2. Materials and methods

2.1. Materials

The assay process involves two discrete incubation steps of 9-minute duration. During the first incubation, 40 μL of sample is incubated with two monoclonal antibodies specific to Aβ (X–42) (biotinylated 21F12) and Aβ (1–X) (ruthenium-labeled 3D6), respectively [15], resulting in formation of a sandwich complex that is specific for the detection of Aβ (1–42). In the second incubation, after addition of streptavidin-coated magnetic microparticles, the sandwich complex becomes bound to the solid phase. For measurement, the reaction mixture is aspirated into a measuring cell where the microparticles are magnetically captured onto the surface of an electrode. Unbound substances are then removed by washing. Application of a voltage to the electrode induces chemiluminescent emission of photons from the ruthenium complex, which are quantified by a photomultiplier.

The assay uses two-point recalibration of a master calibration curve with two supplied lyophilized calibrators in a buffer matrix. The master curve is generated at each lot standardization by Roche Diagnostics and is included in the barcode of the kit. After reconstitution of the lyophilisate, the calibrators are ready to use. Recalibration of an existing reagent pack must be performed if left on board the instrument for >7 days, and recalibration of an existing lot with a new reagent pack must be performed if the last calibration was >4 weeks ago. The assay is run on the cobas e 601 analyzer platform, with a throughput of 170 samples per hour, which was used for all experiments reported here.

Five separate production lots of the final assay composition have been produced and evaluated for the Elecsys β-amyloid (1–42) assay: lots MP01, MP03, P02, P03, and P04. The first lot, MP01, was unavailable for some of the experiments discussed as supplies had been exhausted, whereas the most recent lot, P04, was not included in some experiments as they were completed before availability of this lot.

2.1.1. Substances

The peptides used were as follows. Aβ (1–42) was obtained from R-Peptide (#A-1163-1), Bogart, GA, USA. Aβ (1–38) was obtained from Bachem (H-1194), Bubendorf, Switzerland. Aβ (1–40) was obtained from Sigma-Aldrich (A0189), St. Louis, MO, USA. Drugs used in interference experiments are shown in Supplementary Table 1 and were all obtained from Sigma-Aldrich (with the exception of rivastigmine). Other endogenous substances were obtained from Roche Diagnostics GmbH, Penzberg, Germany. Artificial CSF (aCSF) was prepared according to the method of Jensen et al. [16].

2.2. Methods

2.2.1. Analytical sensitivity

Limit of quantitation (LoQ) was derived according to the method described in Clinical & Laboratory Standards Institute (CSLI) document EP17-A2 [17] for lots MP03, P02, and P03. Four human CSF pools were measured both undiluted and after dilution to concentrations between 2.5 and 20 pg/mL using aCSF as diluent. LoQ was specified as the lowest observed averaged Aβ (1–42) concentration that fulfilled the specification for relative total error of $\leq$30%. For both undiluted and diluted samples, measurements were performed on two different cobas e 601 analyzers with two runs per day on each analyzer over three consecutive days, for a total of 12 runs.

2.2.2. Linearity

Linearity was assessed using four separate dilution series, according to the method described in CLSI EP6-A [18]. Two dilution series were prepared using a human CSF pool, and two dilution series were prepared with aCSF. Further details are provided in Supplementary Methods.

2.2.3. Standardization of the Elecsys β-amyloid (1–42) assay to liquid chromatography–tandem mass spectrometry RMP

The Elecsys β-amyloid (1–42) assay was standardized to the JCTLM approved RMP for quantitation of Aβ (1–42) in human CSF, based on liquid chromatography–tandem mass spectrometry (LC-MS/MS) [19]. Reference standardization was performed with 372 individual human CSF samples measured with both the RMP [19] and the Elecsys β-amyloid (1–42) assay. From these 372 samples, 362 were within the measuring range of the Elecsys β-amyloid (1–42) assay and were, therefore, used in a method comparison. All subsequent assay lots were standardized to a panel of 21 human CSF pools with Aβ (1–42) concentrations ranging from 150 to 2300 pg/mL, with target values that can be traced back to the RMP. This process ensures traceability of each new assay lot back to the RMP.

2.2.4. Lot-to-lot comparison

The comparability of assay lots MP03, P02, and P03 to each other (i.e., MP03 vs. P02, MP03 vs. P03, and P02 vs. P03) was tested using method comparison according to CLSI EP09-A3 [20]. A series of individual human CSF samples (n = 100) with Aβ (1–42) concentrations covering the measuring range of the assay (200–1700 pg/mL) was measured with each assay lot. Comparability of the lots was assessed by correlation and regression analysis [21].

2.2.5. Interference

2.2.5.1. Endogenous substances

The potential for interference of endogenous substances with the Elecsys β-amyloid (1–42) assay was tested according to CLSI EP7-A2 [22] and CLSI I/LA30-A [23] with human CSF pools and aCSF pools spiked with calibrator peptide to reach Aβ (1–42) concentrations in the upper region of the measuring range. The endogenous substances tested were Intralipid (to test for the effect of lipemia), biotin, bilirubin, hemoglobin, rheumatoid factor, human serum albumin, human immunoglobulin (Ig) G, human IgM, human IgA, and human anti-mouse antibodies. Further details are provided in Supplementary Methods.

2.2.5.2. Drugs

The potential for interference of co-administered drugs with the Elecsys β-amyloid (1–42) assay was assessed by measuring Aβ (1–42) concentrations in CSF samples with and without the presence of the potentially interfering drug. Human CSF pools were used in this experiment. A total of 31 drugs tested, listed in Supplementary Table 1, included some frequently administered in the general population, as well as a wider selection of drugs frequently administered in elderly patients at risk for, or experiencing symptoms of, AD. Further details are provided in Supplementary Methods.

2.2.6. Cross-reactivity

The potential for cross-reactivity of the Elecsys β-amyloid (1–42) assay with other amyloid peptides was tested for Aβ (1–38) and Aβ (1–40) according to CLSI EP7-A2 [17]. These two peptides were specifically chosen because they partially share the amino acid sequence of the epitopes of the assay antibody and because they occur more abundantly in CSF than Aβ (1–42) [24]. Three human CSF samples with nominal Aβ (1–42) concentrations of approximately 500, 800, and 1100 pg/mL were spiked with varying concentrations of the two potential cross-reactants (0, 2.5, 5, 10, and 20 ng/mL) before measurement with the Elecsys β-amyloid (1–42) assay. The cross-reactivity percentage was calculated as:

$$\frac{\text{measured concentration of the spiked sample} - \text{known concentration of the nonspiked sample}}{\text{crossreactant concentration}} \times 100$$

Absence of cross-reactivity was established if the cross-reactivity was ≤10%.

2.2.7. Hook effect

To test for the presence of a high-dose hook effect with Aβ (1–42), two dilution series were established with two separate CSF pools (aCSF for lot MP01 and human CSF for lots P02, P03, and P04). The two pools were spiked with calibrator peptide up to a concentration >11,000 pg/mL. For each CSF pool, a dilution series in aCSF was prepared to achieve Aβ (1–42) concentrations of 0–>11,000 pg/mL.

2.2.8. Reagent and calibration stability

Reagent and calibration stability were assessed in a series of experiments that tested the impact of various storage conditions on the reagents. Further details are provided in Supplementary Methods.

Stability of reagents was assessed by comparing Aβ (1–42) recovery for nine separate samples (human CSF pools and aCSF pools spiked with calibrator peptide) with varying Aβ (1–42) concentrations covering the measuring range of the assay (200–1700 pg/mL) and two controls before and after exposure to the conditions and time intervals specified in the Supplementary Methods. Samples and controls were tested in duplicate.

2.2.9. Precision

Repeatability (within-run precision) and intermediate (within-laboratory) precision were assessed according to CLSI EP05 [25] over 21 days with two runs per day and two determinations per run using nine CSF samples (human CSF pools, aCSF pools spiked with calibrator peptide). Intermodule (between-instrument) precision was assessed across four cobas e 601 modules using five CSF samples (human CSF pools, aCSF pools spiked with calibrator peptide).

2.2.10. Multicenter performance evaluation

The multicenter performance evaluation study aimed to assess reproducibility of the Elecsys β-amyloid (1–42) assay under varying conditions (i.e., different sites, different lots, and multiple runs on different days with repetitions). Five different human CSF sample pools covering the measuring range of the assay and two artificial samples (controls) were analyzed to evaluate the external reproducibility. Four different sites (University of Gothenburg, Sweden; VU University Medical Center, Amsterdam, the Netherlands; Washington University at St. Louis, MO, USA; and Covance Indianapolis, IN, USA) and three different assay lots (MP03, P02, and P03) were included in the study, with two sites using all three lots and two sites using two lots. The study design was adapted from CLSI-EP15-A2 [26], with two runs per day for each lot and each sample over 5 days. The total reproducibility and variance components were calculated for each of the samples in terms of coefficient of variation (CV) % by variance components analysis using the analysis of variance type 1 approach for unbalanced data, including the components site, lot, day, run, and within-run precision. Confidence intervals (CIs; 95%) for the total reproducibility were calculated based on the chi-square distribution and Satterthwaite approximation for degrees of freedom.

Each site used one cobas e 601 analyzer and was first familiarized with the instrument and the assay by running the low and high assay controls in 21 repetitions on a single day. In addition, a human CSF pool was run over several days to allow the site to become familiarized with CSF as sample material. Familiarization was followed by a 10-day precision experiment, where each of the two assay controls was measured in two runs per day in duplicate. This experiment was used to set up site-specific quality control rules for the controls, based on site-specific target values and precision values derived from this experiment.

3. Results

3.1. Analytical sensitivity

Target Aβ (1–42) concentrations, measured Aβ (1–42) concentrations, total error, and relative total error for undiluted and diluted samples were determined for the four separate CSF samples tested with MP03, P02, and P03 lots. Based on these results, the LoQ for Aβ (1–42) was calculated as 5.10 pg/mL for P03, 10.64 pg/mL for MP03, and 11.28 pg/mL for P02. Data from P02, the lot with the highest LoQ, are listed in Table 1. The overall LoQ value across the three lots was $\leq$11.28 pg/mL.

3.2. Linearity

Linearity was demonstrated throughout the measuring range (200–1700 pg/mL) for all four dilution series. These results were consistent across the three separate assay lots MP01, P02, and P03.

Table 1
Target and measured concentrations, and total error, for CSF samples in limit of quantitation experiment for lot P02

Sample number	Dilution level (relation to original sample %)	Target concentration (pg/mL)	Mean measured concentration (pg/mL)	SD (pg/mL)	Bias (pg/mL)	Total error (pg/mL)	Relative total error (%)
CSF1	100	171	175.4	7.53	4.42	8.73	5.10
	2.92	5	1.22	1.20	3.78	3.97	79.38
	8.77	15	9.84	1.15	5.16	5.29	35.25
CSF2	100	175	175.5	4.40	0.50	4.43	2.53
	2.86	5	1.72	1.51	3.29	3.61	72.27
	8.57	15	11.67	1.33	3.33	3.59	23.92
CSF3	100	198	196.75	5.46	1.25	5.60	2.83
	2.53	5	1.30	1.19	3.70	3.89	77.72
	7.58	15	11.28	1.18	3.73	3.91	26.05
CSF4	100	152	163.0	4.16	11.0	11.76	7.74
	3.29	5	1.87	1.17	3.13	3.34	66.84
	9.87	15	11.32	0.89	3.68	3.79	25.27

Abbreviations: CSF, cerebrospinal fluid; SD, standard deviation.

3.3. Standardization of the Elecsys β-amyloid (1–42) assay to LC-MS/MS reference method

Fig. 1 shows the method comparison between the Elecsys β-amyloid (1–42) method and the LC-MS/MS reference method within the measuring range (n = 362 samples). The correlation between the two methods (Pearson's r) was 0.93.

3.4. Lot-to-lot comparison

The method comparison for the lot-to-lot comparability experiments is shown in Fig. 2A–C. Excellent comparability in terms of correlation and absolute values was observed between all lots. For lot MP03 versus lot P02, the intercept and slope (Passing-Bablok linear regression analysis) were 9.76 (95% CI, 4.94–15.7) and 0.996 (95% CI, 0.985–1.00). For lot MP03 versus lot P03, the intercept and slope were 18.3 (95% CI, 11.7–24.6) and 0.984 (95% CI, 0.972–0.994). For lot P02 versus lot P03, the intercept and slope were 6.81 (95% CI, 0.993–15.9) and 0.987 (95% CI, 0.973–1.00). The correlation coefficients (Pearson's r) were 0.998 (lot MP03 vs. lot P02), 0.996 (lot MP03 vs. lot P03), and 0.997 (lot P02 vs. lot P03). The proportional bias between all lots was <2% in the medical decision area.

3.5. Interference

3.5.1. Endogenous substances

No interference was observed with any of the three CSF pools tested up to the highest concentrations of the endogenous substances (data not shown).

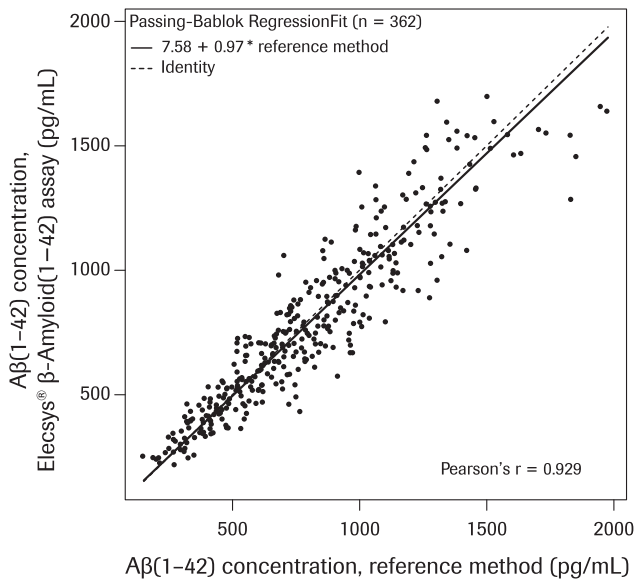

Fig. 1. Method comparison after standardization of the Elecsys Aβ (1–42) assay to the liquid chromatography–tandem mass spectrometry reference method (Leinenbach et al.) [18].

3.5.2. Drugs

Interference of drugs was within specifications for all drugs with the lower drug concentration tested (C1) for any of the three samples. Interference of drugs was also within specifications for the higher drug concentration tested (C2) for any of the three samples except for cefoxitin (87.7% recovery at a drug concentration of 2500 mg/L) and metformin (88.2% recovery at a drug concentration of 12 g/L).

3.6. Cross-reactivity

No significant cross-reactivity was observed with either Aβ (1–38) (cross-reactivity was −0.17% to −1.92% with the 500-pg/mL sample, −0.24% to −1.88% with the 800-pg/mL sample, and −0.42% to −1.72% with the 1100-pg/mL sample) or Aβ (1–40) (cross-reactivity was −0.40% to −1.12% with the 500-pg/mL sample, −0.57% to −1.36% with the 800-pg/mL sample, and −0.76% to −2.76% with the 1100-pg/mL sample).

3.7. Hook effect

Across two separate dilution series for lot MP01, no hook effect was observed up to 9000 pg/mL and beyond, which exceeds the upper end of the measuring range (1700 pg/mL) by more than fivefold (Supplementary Fig. 1). This result was reproduced in analysis of three further lots (P02, P03, and P04).

3.8. Reagent and calibrator stability

The stability of reagents and calibrations exceeded specifications for all stability experiments. On-board calibration frequency exceeded 7 days and lot calibration frequency exceeded 4 weeks. On-board reagent stability was found to be >4 weeks, whereas reagent stability at 2°C–8°C after first opening was >8 weeks. Transport stability exceeded 1 week at 25°C.

3.9. Precision

Repeatability CVs for human CSF pools were 1.0%–1.6% and intermediate CVs were 1.9%–4.0% (Table 2). Intermodule CVs were 1.1%–3.9% (Table 3).

3.10. Multicenter performance evaluation

The five samples and two controls analyzed showed an estimated total reproducibility (including all sources of variability—site, lot, day, run, and within-run; %CV) ranging between 2.0% and 5.1% (Table 4). The estimated intersite precision was ≤3.5% for all samples and the intermediate (within-laboratory) precision ranged between 1.7% and 3.6% for all samples. The estimated repeatability (within-run precision) was ≤2.7% for all samples. The estimated lot-to-lot variability was ≤2.3% for all samples.

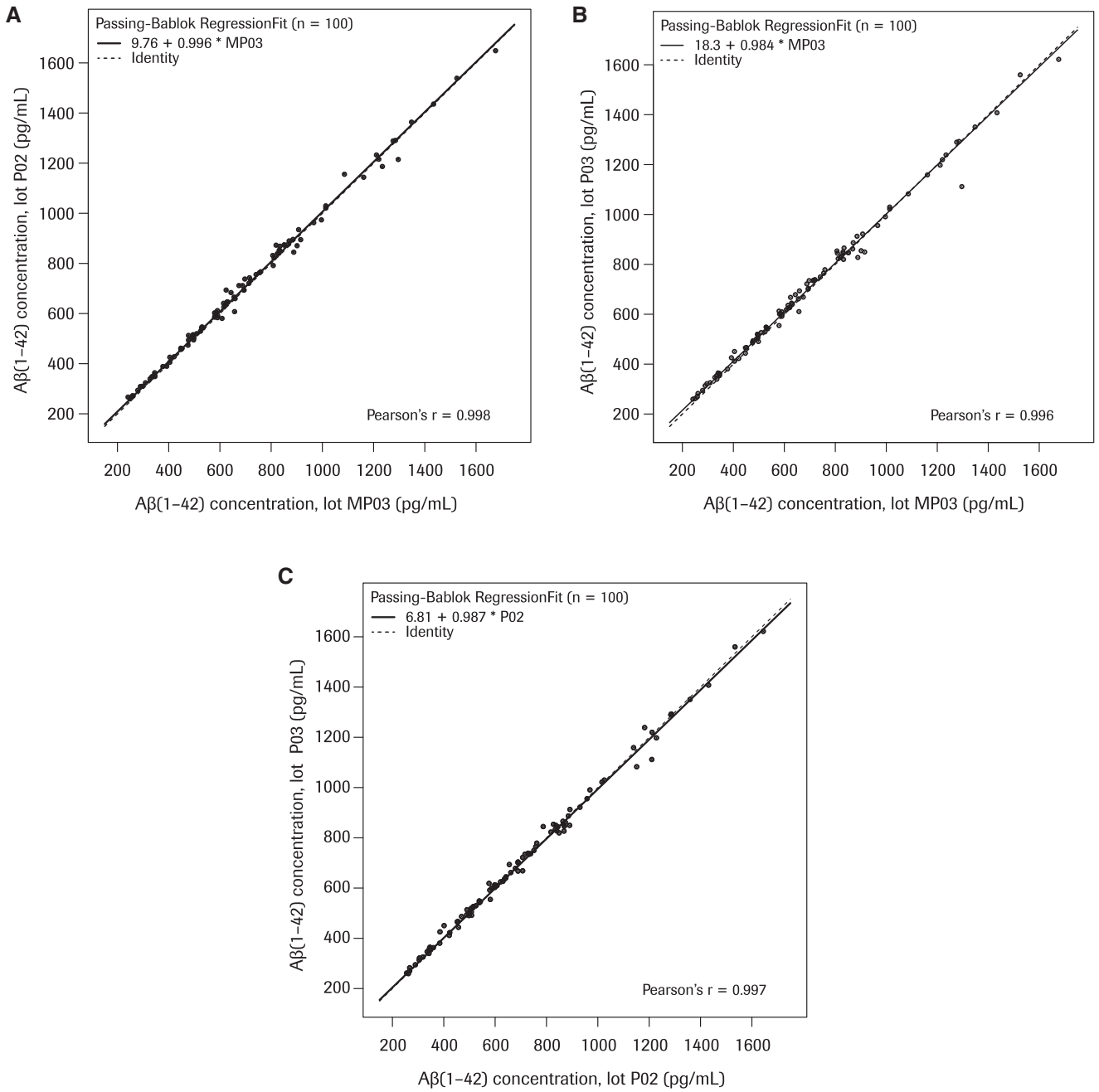

Fig. 2. Comparison between assay lots for Elecsys Aβ (1–42). (A) Lot P02 versus lot MP03. (B) Lot P03 versus lot MP03. (C) Lot P02 versus lot P03.

4. Discussion

Here, we describe the results of technical performance experiments for a novel electrochemiluminescence assay for the quantitation of Aβ (1–42) in human CSF. The Elecsys β-amyloid (1–42) assay demonstrates low between-laboratory and lot-to-lot variability and is standardized to an JCTLM approved reference method [19], implying that use of this assay might allow for establishment of cutoff concentrations with global applicability and consistency over time.

The total reproducibility with the Elecsys β-amyloid (1–42) assay over four different sites ranged between 2.0% and 5.1%. By comparison, available assays for CSF Aβ (1–42) demonstrate considerable variability between laboratories and between different lots. Results from the Alzheimer's Association Quality Control (AAQC) program (rounds 1–9, 2010–2012) showed that overall variability for Aβ (1–42) analysis was 17%–29% for INNOTEST ELISA, 17%–38% for INNO-BIA AlzBio3, and 13%–36% for Meso Scale Diagnostic (MSD) [13]. The improved variability of the Elecsys β-amyloid (1–42) assay in comparison with commercially available assays has been confirmed in the latest rounds of the AAQC program, which include the

Table 2
Repeatability and intermediate precision

Sample ID	Mean measured concentration (pg/mL)	Repeatability (within-run precision)		Intermediate precision (within-laboratory)	
		SD (pg/mL)	%CV (upper limit of 95% CI)	SD (pg/mL)	%CV (upper limit of 95% CI)
Low control	470	3.66	0.8 (1.0)	7.614	1.6 (2.1)
High control	978	8.45	0.9 (1.1)	15.93	1.6 (2.1)
CSF1	249	2.91	1.2 (1.5)	5.14	2.1 (2.6)
CSF2	433	6.08	1.4 (1.8)	11.47	2.6 (3.3)
CSF3	619	6.79	1.1 (1.4)	15.56	2.5 (3.1)
CSF4	767	9.50	1.2 (1.6)	22.08	2.9 (3.6)
CSF5	816	8.01	1.0 (1.2)	15.30	1.9 (2.3)
CSF6	873	10.90	1.2 (1.6)	18.37	2.1 (2.6)
CSF7	931	11.64	1.3 (1.6)	36.87	4.0 (5.0)
CSF8	1069	12.26	1.1 (1.5)	21.03	2.0 (2.4)
CSF9	1456	23.01	1.6 (2.0)	37.60	2.6 (3.2)

Abbreviations: SD, standard deviation; CV, coefficient of variation; CI, confidence interval; CSF, cerebrospinal fluid.

Elecsys β-amyloid (1–42) assay. Overall variability in round 16 was 17%–27% for INNOTEST ELISA, 30%–40% for INNO-BIA AlzBio3, 11%–13% for MSD, and 19%–57% with EuroImmune/ADx, compared with 2.5%–3.0% for the Elecsys β-amyloid (1–42) assay [27]. In round 17, overall variability was 14%–19% for INNOTEST ELISA, 15%–17% for INNO-BIA AlzBio3, 20%–21% for MSD, and 6.5%–8.2% with EuroImmune/ADx, compared with 1.9%–3.2% for the Elecsys β-amyloid (1–42) assay [28].

Notably, the Elecsys β-amyloid (1–42) assay has high lot consistency—variability due to lot effect in the multicenter performance evaluation study [CV] was $\leq$2.3%, and the lot-to-lot method comparison experiments showed a bias of $<$2% in the medical decision area and correlation coefficients (Pearson's r) of $>$0.99 for all three assay lots. The reduced lot-to-lot variability with the Elecsys β-amyloid (1–42) assay arises from the rigid lot standardization process, which involves comparison with a panel of native samples with target values traceable to an JCTLM approved RMP [19]. Lack of standardization of current assays to a certified reference material or RMP is one of the principal unmet needs in the quantification of Aβ (1–42) in CSF [14,29]. Without standardization, the apparent concentration of the biomarkers measured is likely to vary between assays, leading to difficulties with interpretation and comparison of results between laboratories using different assays, and thereby preventing introduction of universal cutoff concentrations to aid in the diagnosis of AD [14]. Universal cutoff concentrations are already applied for several biomarkers in clinical routine, including HbA1c in diabetes mellitus [30]. The next step would be to apply the same concept also to Aβ (1–42) in CSF in patients with AD.

Table 3
Intermodule precision

Sample ID	Mean measured concentration (pg/mL)	SD (pg/mL)	%CV
Low control	466	2.20	0.5
High control	977	7.60	0.8
CSF1	120	3.42	2.9
CSF2	247	3.60	1.5
CSF3	455	17.82	3.9
CSF4	670	7.65	1.1
CSF5	913	27.13	3.0

Abbreviations: SD, standard deviation; CV, coefficient of variation; CSF, cerebrospinal fluid.

Our results demonstrate that the Elecsys β-amyloid (1–42) assay is robust, with good analytical sensitivity and linearity over the desired measuring range with no high-dose hook effect. Furthermore, the assay demonstrated good specificity, with no cross-reactivity for Aβ (1–38) or Aβ (1–40). No interference of endogenous substances with the assay was noted, and of the large number of drugs tested in interference experiments, only cefoxitin and metformin exhibited any interference with the assay. Furthermore, the drug interferences were noted at concentrations much higher than expected therapeutic levels (cefoxitin 2500 mg/L, expected therapeutic level in CSF: 2.8 mg/L [31]; metformin 12 g/L, expected therapeutic level in plasma: 1–2 mg/L [32]).

A potential limitation of this study, which remains applicable to all studies using currently available immunoassays, is the absence of a robust preanalytical sample handling procedure. The principal variables that have been demonstrated to have significant effects on measured concentrations of Aβ (1–42) in CSF samples are tube material (polypropylene and polystyrene) and CSF storage conditions (ambient temperature, number of freeze/thaw cycles before analysis) [33,34]. Although guidelines have been proposed to standardize preanalytical procedures for AD biomarkers [34], these are not yet universally adopted but are vital for introduction of universal cutoffs.

As discussed, diagnosis based on CSF Aβ (1–42) measurements is not yet widely performed in routine clinical practice, partly because of issues with available assays. An alternative is PET imaging with amyloid-specific radiotracer ligands, which is now an established diagnostic tool, as three PET tracers are currently approved by the US

Table 4
Total reproducibility

Sample	Mean concentration (pg/mL)	Reproducibility*, %CV (95% CI)	Between-site variability, %CV	Lot-to-lot variability, %CV	Intermediate precision†, %CV (95% CI)	Between-day, %CV	Between-run, %CV	Repeatability (within-run), %CV (95% CI)
Low control	486	2.0 (1.7–2.5)	0.7	0.6	1.8 (1.5–2.1)	1.4	0.5	1.0 (0.9–1.2)
High control	869	2.2 (1.7–3.1)	0.8	1.0	1.7 (1.5–2.1)	1.4	0.5	0.9 (0.8–1.1)
CSF1	342	3.3 (2.2–6.0)	0	2.3	2.4 (2.1–2.7)	1.8	0.2	1.6 (1.4–1.8)
CSF2	662	2.6 (2.0–3.9)	0.5	1.5	2.1 (1.9–2.5)	1.4	0.8	1.4 (1.2–1.6)
CSF3	755	5.1 (3.6–8.3)	3.5	0.6	3.6 (3.3–4.1)	0	2.5	2.7 (2.3–3.1)
CSF4	935	2.2 (1.8–2.9)	0.8	0.9	1.9 (1.7–2.2)	1.0	0.7	1.5 (1.3–1.7)
CSF5	1472	3.2 (2.5–4.5)	1.8	0.8	2.6 (2.3–3.0)	1.6	0.2	2.0 (1.8–2.4)

Abbreviations: CV, coefficient of variation; CI, confidence interval; CSF, cerebrospinal fluid.
*Total variability, includes all factors (estimated from a variance components model): site, lot, day, run, and within-run.
†Intermediate precision includes between-day, between-run, and within-run precision.

Food and Drug Administration (FDA) for clinical diagnostic use. However, PET imaging has potential limitations, including high cost, requirement for a PET scanning instrument at the clinical center, and short half-lives of the (18F) radiotracers used (requiring either a cyclotron at or near the PET scanning center or rapid transport of the radiotracers to the center from elsewhere [35]). Nevertheless, the FDA approval of amyloid PET tracers [36–38] shows the medical need for robust, reliable, and widely available biomarkers to determine the presence of amyloid pathology in clinical routine.

A frequently cited obstacle to the use of CSF as a source for AD biomarkers is that obtaining samples from individuals requires a lumbar puncture (LP) procedure, around which there are a number of negative perceptions of potential complications, such as post-LP headache. In fact, a number of studies over almost 20 years have shown that LP is associated with mild headache in <5% of subjects [39–42], a frequency only slightly greater than is observed after amyloid PET (≤1%–1.8%) [36,37,43]. Furthermore, recent clinical trials of anti-amyloid immunotherapies have increasingly moved toward using LP to obtain CSF samples for analysis of biomarkers [44–46]. In some countries, the procedure is not yet routine and physicians may require training and experience to perform CSF collection.

The potential availability of DMTs in coming years creates the need for reliable diagnostic tests to accurately identify patients with amyloid pathology who are likely to benefit most from these agents. Improved diagnostic tests will also enrich clinical trial populations with the appropriate, amyloid-positive patients, thereby increasing the likelihood that further DMTs will emerge. Along with amyloid PET imaging, CSF Aβ (1–42) testing is now regarded as an essential pillar of AD diagnosis to identify amyloid pathology as the underlying cause of symptoms [4,47]. Furthermore, the combination of CSF tau proteins (total and phosphorylated tau) with Aβ (1–42) is a sensitive and specific predictor of progression from MCI to AD [5,6] and has the potential to assist with differential diagnosis of AD from other causes of dementia, such as Creutzfeldt-Jakob disease [48,49], frontotemporal dementia, and Lewy body dementia [50]. Given the weight of evidence supporting the utility of CSF Aβ (1–42) in AD diagnosis, the only significant barrier remaining to routine clinical use is the variability in measured Aβ (1–42) levels [51], which results mainly from limitations of available assays. Further evaluation of individual assays in prospective, longitudinal clinical trials will strengthen confidence in their use, but such trials are not necessary at this point to establish the clinical utility of the biomarkers themselves.

The results described here provide evidence that the novel Elecsys β-amyloid (1–42) assay embodies many of the desired attributes for an ideal assay, including marked improvements in precision compared with available

assays, even between laboratories. Part of the improved performance is certainly because of the fully automated procedure on the cobas platform. In addition, the assay is the first CSF Aβ (1–42) assay standardized to an JCTLM approved RMP, and all assay lots are standardized to a sample set with target values derived from the RMP, providing good lot-to-lot comparability. In conclusion, results from the Elecsys β-amyloid (1–42) assay will be highly replicated across laboratories and lots. This represents a fundamental prerequisite for the establishment of a robust cutoff value that is valid worldwide now and into the future.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalz.2015.09.009.

RESEARCH IN CONTEXT

1. Systematic review: We reviewed the literature using PubMed and incorporated searches of conference abstracts and assay datasheets. Available data on the technical performance of alternative assays for quantitation of amyloid-beta 1–42 (Aβ [1–42]) in human cerebrospinal fluid (CSF) have been appropriately reviewed and cited.
2. Interpretation: In our study, we have demonstrated that a novel, fully automated assay for quantitation of Aβ (1–42) in human CSF has excellent reproducibility, including high lot-to-lot comparability. These measures contrast with those of commercially available assays for Aβ (1–42), suggesting that this assay might improve the precision of Aβ (1–42) quantitation in CSF. Furthermore, the assay is the first to be standardized to a candidate reference measurement procedure.
3. Future directions: Further research is required in the clinical setting to evaluate a cutoff value with this assay for Aβ (1–42) that will guide its potential use in the diagnostic setting in AD.

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